基础研究

Anti-miR-145促进人气道平滑肌细胞增殖及骨桥蛋白合成

陈培芬[1],邱智辉[2],黄国华[3],张香梅[4],彭武建[1],曾 辉[1],赖文岩[5]
广东深圳市第三人民医院[1]内二科,[2]内一科,[4]病理科,广东 深圳 518112;南方医科大学附属南方医院[3]呼吸科,[5]心内科实验室,广东 广州 510515

摘要:目的 观察miR-145抑制剂(Anti-miR-145)对人支气管平滑肌细胞(HASMCs)的影响,探讨其在哮喘气道重塑中的作用。**方法** 分为对照组和实验组,实验组加入不同浓度的Anti-miR-145(10~100 nmol/L)。CCK-8法检测细胞增殖,流式细胞术检测细胞凋亡,Western blotting 检测骨桥蛋白合成。**结果** 与对照组比较,10 nmol/L 和50 nmol/L Anti-miR-145显著促进HASMCs增殖及骨桥蛋白合成($P<0.05$或$P<0.01$),50 nmol/L Anti-miR-145 显著抑制HASMCs 凋亡($P<0.01$)。**结论** Anti-miR-145通过刺激HASMCs增殖和骨桥蛋白合成,抑制其凋亡,可能在哮喘气道重塑中发挥重要作用。
关键词:anti-miR-145;气道平滑肌细胞;增殖;骨桥蛋白

Anti-miR-145 promotes human airway smooth muscle cell proliferation and osteopontin synthesis *in vitro*

CHEN Peifen[1], QIU Zhihui[2], HUANG Guohua[3], ZHANG Xiangmei[4], PENG Wujian[1], CENG Hui[1], LAI Wenyan[5]
[1]Second Department of Internal Medicine, [2]First Department of Internal Medicine, [4]Department of Pathology, Third People's Hospital of Shenzhen, Shenzhen 518112, China; [3]Department of Respiratory Diseases, [5]Laboratory of Cardiology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China

Abstract: Objective To investigate the effect of anti-miR-145 on human airway smooth muscle cell (HASMC) proliferation and osteopontin systhesis *in vitro* and explore the mechanisms. **Methods** HASMCs were treated with 10-100 nmol/L anti-miR-145, and the cell proliferation and apoptosis were investigated using a CCK-8 assay and flow cytometry, respectively. The changes in osteopontin synthesis after the treatment was quantified with Western blotting. **Results** Treatment with 10 and 50 nmol/L anti-miR-145 significantly promoted the proliferation and osteopontin synthesis in HASMCs ($P<0.05$ or <0.01), and 50 nmol/L anti-miR-145 obviously inhibited the cell apoptosis ($P<0.01$). **Conclusion** Anti-miR-145 promotes HASMC proliferation and osteopontin synthesis and inhibits HASMC apoptosis *in vitro*, indicating the important role of anti-miR-145 in the pathogenesis of airway remodeling.
Key words: anti-miR-145; airway smooth muscle cells; proliferation; osteopontin

气道重塑是支气管哮喘的基本特征之一。哮喘气道平滑肌细胞数量增加、肥大是气道重塑的重要原因[1]。miR-145是一种22-nt的高度保守的微小RNA(miRNA)。研究发现miR-145通过促进肿瘤细胞生长[2]、凋亡[3],但其是否能影响人支气管平滑肌细胞(HASMCs)增殖与凋亡,国内外未见报道。本研究拟探讨miR-145对ASMCs的增殖、凋亡的影响以初步了解其在哮喘中的作用。

1 材料和方法

1.1 材料

DMEM培养基(Gibco),胎牛血清(PAA),CCK-8增殖试剂盒、Annexin V-FITC细胞凋亡检测试剂盒(北京碧云天公司),Lipofectamine 2000(Invitrogen),α-actin、骨桥蛋白单克隆抗体(武汉博士德),Anti-miR-145(Applied Biosystems),流式细胞仪(美国BD)。

1.2 HASMCs原代培养传代,同文献[4]

以平滑肌特异的α-actin 单克隆抗体免疫荧光染色,鉴定为ASMCs。

1.3 转染

将ASMCs细胞分为对照组和实验组,对照组不加Anti-miR-145,实验组加入不同浓度的Anti-miR-145(10、50、100 nmol/L)。转染在六孔板内,待细胞结合度约50%~70%时进行,加入不同浓度的Anti-miR-145和10 μL的Lipofectamine 2000。转染后5 h换液。

1.4 CCK-8检测ASMC的增殖活力

转染5 h后,每孔加入CCK-8 10 μL,继续37 ℃培养2 h,酶联免疫检测仪上450 nm处检测吸光度(A_{450}值)。具体同文献[5]。

收稿日期:2014-08-22
基金项目:深圳市科技计划(201102064)
作者简介:陈培芬,副主任医师,E-mail: drcpf@sohu.com

1.5 流式细胞术检测ASMCs凋亡

上述细胞制成2×10⁵/mL的悬液,取0.5 mL离心,195 μL Annexin V-FITC结合液重悬,加入5 μL Annexin V-FITC混匀避光孵育15 min。离心弃上清,再次以195 μL Annexin V-FITC结合液重悬,加10 μL氯化丙啶,流式细胞仪检测。凋亡率=阳性细胞数/细胞总数。

1.6 Western blotting检测Anti-miR-145转染对ASMCs骨桥蛋白(OPN)合成的影响。同文献[6]。

1.7 统计学处理

采用SPSS 13.0统计分析,数据以均数±标准差表示。组间比较采用单因素方差分析,两两比较采用LSD法。

2 结果

2.1 培养的HASMCs及鉴定

倒置相差显微镜下,HASMCs汇合后呈典型的"峰、谷"状。α-actin免疫荧光染色见绿色荧光。

2.2 Anti-miR-14对HASMCs增殖的影响

10 nmol/L和50 nmol/L anti-miR-145转染HASMC后,平均吸光度值显著高于对照组。其中10 nmol/L组为0.986±0.103,较对照组0.834±0.128显著增高(P<0.05);50 nmol/L组为1.101±0.089,较对照组显著增高(P<0.01);而100 nmol/L anti-miR-145抑制剂转染ASMC后,平均吸光度0.932±0.115与对照组比较无显著差异(P>0.05)。

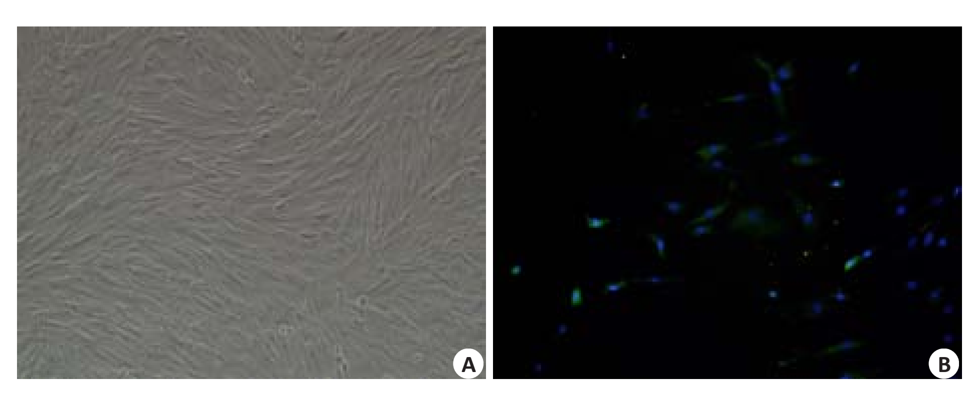


图1 培养的HASMCs形态及鉴定

Fig.1 Morphological and phenotypic identification of cultured HASMCs (Original magnification:×100). *A*: The typical peak and valley growth pattern of HASMCs; *B*: Immunofluorescence staining for α-SMA (green-fluorescence).

2.3 Ani-miR-145对HASMCs凋亡的影响

如图2所示,50 nmol/L anti-miR-145组凋亡率(3.57±0.35)%较对照组(6.4±0.36)%显著降低(P<0.01)。提示Anti-miR-145抑制HASMCs凋亡。

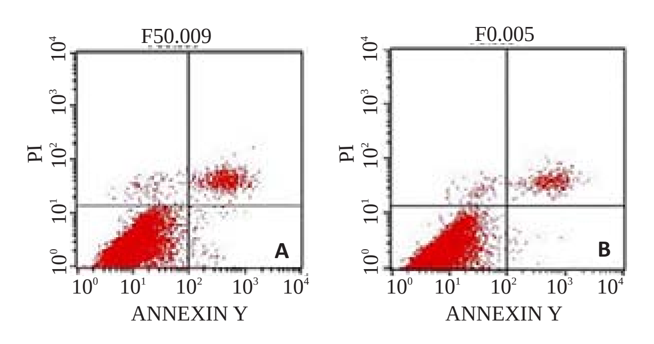


图2 Anti-miR-145对HASMCs凋亡的影响

Fig.2 Effect of anti-miR-145 on HASMC apoptosis. *A*: Control group; *B*: 50 nmol/L anti-miR-145 group.

2.4 Ani-miR-145对骨桥蛋白合成的影响

如图3所示,10 nmol/L、50 nmol/L anti-miR-145促进HASMCs骨桥蛋白合成。100 nmol/L对HASMCs骨桥蛋白合成无明显影响。

3 讨论

miR-145是一种22-nt的高度保守miRNA,作用于c-Myc、CDK4进而抑制细胞增殖,使其停止在G1/S期[7],并通过Akt(P13K/Akt信号转导途径)、ERK(细胞外信号调节激酶(ERK)、EGFR(EGFR/MAPK信号通路)和NUDT1途径抑制细胞生长[8]。且miR-145可通过抑制DEF45蛋白的表达,而诱导结肠癌细胞凋亡[3]。已在肺组织中发现miR-145表达[9]。Collison等[10]发现,哮喘小鼠气道平滑肌miR-145表达上调,拮抗miR-145可减轻哮喘小鼠气道嗜酸性粒细胞炎症、气道粘液分泌、Th2细胞因子产生和气道高反应,其治疗效果可与激素媲美,提示miR-145可能在哮喘气道炎症中具有重要作用。但miR-145对哮喘气道重塑的关键细胞-ASMCs是否同样存在影响,国内外均未见相关的报道。本研究结果表明,拮抗miR-145可促进HASMCs增殖,抑制其凋亡,提示miR-145可能通过抑制平滑肌细胞增殖、促进其凋亡而参与支气管哮喘气道重塑。

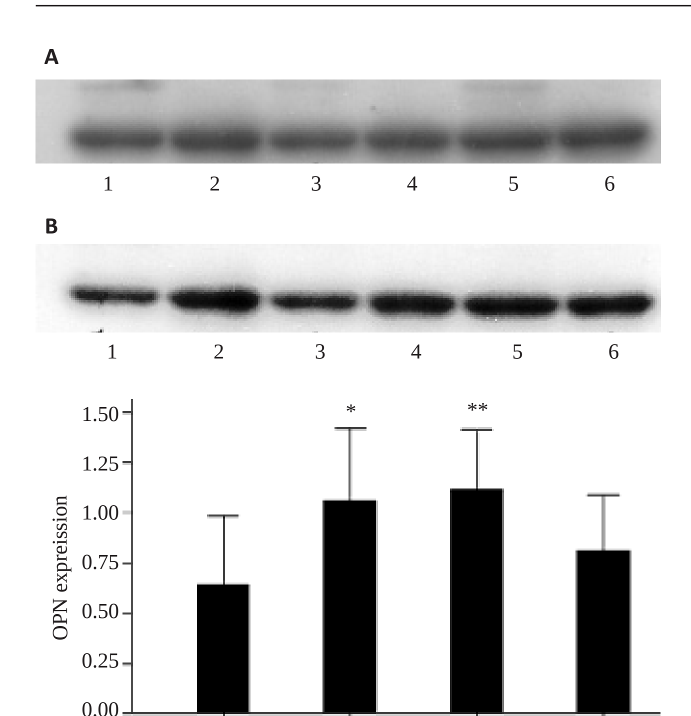


图3 不同浓度Ani-miR-145对骨桥蛋白合成的影响

Fig.3 Anti-miR-145 enhances osteopontin (OPN) synthesis in HASMCs. Upper: Western blotting results. HASMCs were stimulated with DMEM (control) and anti-miR-145 at 10, 50, and 100 nmol/L (Lanes 2, 3, and 4, respectively) for 48h. Lower: Quantitative analysis of the results (*Mean*±*SD*, *n*=3). **P*<0.05, ***P*<0.01 *vs* control.

Simoes等[11]和Kohan等[12-13]通过哮喘小鼠相继证实，与WT组比较，OPN-/-小鼠气道气道平滑肌增生面积减少、上皮下沉积的胶原蛋白含量降低。其研究尚发现重组OPN能促进支气管平滑肌细胞增殖分化。此外，有研究发现OPN可通过c-Myc[14]、ERK[15]通路促进细胞增殖。鉴于本研究发现Anti-miR-145可促进HASMCs增殖，我们进一步探讨了miR-145与OPN的关系。研究结果表明，Anti-miR-145促进OPN蛋白合成，提示miR-145促进HASMCs增殖可能与OPN有关。

总之，anti-miR-145可促进HASMCs增殖，抑制其凋亡，其机制可能与通过上调OPN表达有关。miR-145可能在支气管哮喘气道重塑中起重要作用。

参考文献：

［1］Johnson PR, Roth M, Tamm M, et al. Airway smooth muscle cell proliferation is increased in asthma［J］. Am J Respir Crit Care Med, 2001, 164(3): 474-7.

［2］Zhong M, Ma X, Sun CJ, et al. MicroRNAs reduce tumor growth and contribute to enhance cytotoxicity induced by gefitinib in non-small cell lung cancer［J］. Chem Biol Interact, 2010, 184(3): 431-8.

［3］Zhang J, Guo H, Qian G, et al. MiR-145, a new regulator of the DNA Fragmentation Factor-45(DFF45)-mediated apoptotic network［Z］, 2010: 211.

［4］高 杨, 钟浩海, 罗雅玲, 等. PTEN基因表达改变对人气道平滑肌迁移的影响［J］. 南方医科大学学报, 2011, 31(3): 403-8.

［5］陈培芬, 罗雅玲, 赖文岩, 等. 巨噬细胞移动抑制因子促进肺成纤维细胞增殖和胶原合成［J］. 中国病理生理杂志, 2009, 25(4): 699-702.

［6］陈培芬, 罗雅玲, 赖文岩, 等. 巨噬细胞移动抑制因子经Rho途径促进人肺成纤维细胞Ⅰ型胶原合成［J］. 医学理论与实践, 2011, 27(14): 1621-4.

［7］Chen Z, Zeng HZ, Guo Y, et al. miRNA-145 inhibits non-small cell lung cancer cell proliferation by targeting c-Myc［J］. J Exp Clin Cancer Res, 2010, 29(8): 151.

［8］Zhong M, Ma X, Sun C, et al. MicroRNAs reduce tumor growth and contribute to enhance cytotoxicity induced by gefitinib in non-small cell lung cancer［J］. Chem Biol Interact, 2010, 184(3): 431-8.

［9］Boettger T, Beetz N, Kostin S, et al. Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the Mir143/145 gene cluster［J］. J Clin Invest, 2009, 119(9): 2634-47.

［10］Collison A, Mattes J, Plank M, et al. Inhibition of house dust mite-induced allergic airways disease by antagonism of mircoRNA-145 is comparable to glucocorticoid treatment［J］. J Allergy Clin Immunol, 2011, 128(1): 160-7.

［11］Simoes DC, Xanthou G, Petrochilou KA, et al. Osteopontin deficiency protects against airway remodeling and hyperresponsiveness in chronic asthma［J］. Am J Respir Crit Care Med, 2009, 179(10): 894-902.

［12］Kohan M, Breuer R, Berkman N, et al. Osteopontin induces airway remodeling and lung fibroblast activation in a murine model of asthma［J］. Am J Respir Cell Mol Biol, 2009, 41(3): 290-6.

［13］Kohan M, Bader R, Puxeddu I, et al. Enhanced osteopontin expression in a murine model of allergen-induced airway remodelling［J］. Clin Exp Allergy, 2007, 37(10): 1444-54.

［14］Martinez C, Churchman M, Freeman T, et al. Osteopontin provides early proliferative drive and May be dependent upon aberrant c-myc signalling in murine intestinal tumours［J］. Exp Mol Pathol, 2010, 88(2): 272-7.

［15］Yu HW, Liu QF, Liu GN. Positive regulation of the Egr-1/osteopontin positive feedback loop in rat vascular smooth muscle cells by TGF-beta, ERK, JNK, and p38 MAPK signaling［J］. Biochem Biophys Res Commun, 2010, 396(2): 451-6.

（编辑：孙昌朋）